STUDIES ON LIBERATION OF ACETYL CHOLINE IN THE SKIN*

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(Received for publication October 6, 1939)

It has been shown repeatedly that cutaneous stimuli such as intradermal injections (1, 2), application of heat (3) and ultraviolet light (4) produce generalized stimulation of the parasympathetic nervous system. This phenomenon was called the "vagal reflex" (5). In the light of newer research work this phenomenon might be related to the liberation of acetyl choline since it is known that any parasympathetic impulse becomes effective at the nerve endings as a result of liberation and action of acetyl choline. One might suppose, for example, that the fall of blood pressure in dermatitis due to ultraviolet light (4) is caused by continuous liberation of acetyl choline from the inflamed skin. There is much presumptive evidence for a local liberation of acetyl choline in the skin, especially if hyperemia follows the cutaneous stimulation. It was supposed (6) that the erythematous flare around histamine wheals is caused by liberation of acetyl choline (cf. 11). Some experiments (7) seemed to indicate that any injury of the skin, even the slightest one, is followed by the local liberation of this substance. The experiments described in another paper appearing in this issue of the JOURNAL support the assumption of such a mechanism.

With the recently improved methods for detecting acetyl

* From the Section of Dermatology of the Department of Medicine, and the Department of Pharmacology, University of Chicago. Read before the Second Annual Meeting of the Society for Investigative Dermatology, Inc., St. Louis, Missouri, May 16, 1939.

This work was aided in part by grants from the Commonwealth Fund, and the Dr. Wallace C. and Clara A. Abott Memorial Fund of the University of Chicago.

choline in blood and tissues, we endeavoured to demonstrate its presence in the skin of cats and dogs after experimental local cutaneous stimulation; and in the blood coming from the stimulated skin area in human beings and in animals.

I. EXPERIMENTS WITH BLOOD AND SKIN EXTRACTS AFTER CUTANEOUS STIMULI

Method

For cutaneous stimulation hot and cold water, ultraviolet light, and intracutaneous injections of saline, aolan, and histamine were used in cats, dogs, and human beings with many variations in dosage. In animal experiments skin and outflowing venous blood, and in man venous blood only, were tested for the presence of acetyl choline immediately after stimulation and several times during the following forty-eight hours.

Acetyl choline after its liberation and its action on the effector organ is split very quickly by the acetyl choline esterase of the tissues and of the blood into acetic acid and the relatively inactive choline. The hydrolytic action of the choline esterase is inhibited by physostigmine. Hence in order to demonstrate liberation of acetyl choline in the tissues or blood, toxic doses of physostigmine (eserine) must be administered.

In human beings the required high doses of physostigmine could not be administered. Therefore, in human experiments the blood was drawn into syringes containing the required amount of physostigmine and heparin, the latter in order to avoid coagulation (0.5 mgm. physostigmine and 5–10 mgm. heparin for each 10 cc. of blood). In addition, 0.5 mgm. physostigmine (or the similarly acting prostigmine) was given subcutaneously shortly before the cutaneous stimulation.

In experiments on cats and dogs (1) large doses of physostigmine could be given (1–3 mgm. per kg. body weight); (2) rough stimuli could be applied; (3) the circulation of the extremity treated with cutaneous stimuli could be arrested for a long time in order to concentrate the acetyl choline in the blood coming from the stimulated area; (4) heparin could be given intravenously, and (5) large pieces of skin could be excised.

In cats and dogs, the skin was shaved after physostigmine and heparin were given intravenously. The circulation was arrested in one extremity, and the blood was taken from the large vein of this extremity before and after cutaneous stimulation.

For experiments with skin extracts a large area of the shaved abdomen was used on one side for stimuli, on the other side for control. The skin excisions were made immediately after cutaneous stimulation and later after varying intervals. For extraction of the skin the method of Graeser, Ginsberg and Friedemann (8) was used. Immediately after excision the skin samples were frozen in liquid nitrogen and ground to a powder in the freezing apparatus described by these authors. The frozen skin powder was placed in 10% trichloracetic acid and treated according to the method of Chang and Gaddum (9). With this

treatment acetyl choline is obtained in a protein-free aqueous solution which is convenient for biological tests.

Blood and skin extracts were tested for acetyl choline on the blood pressure of cats before and after atropinisation and on the sensitized leech muscle preparation (10). The latter is sensitive to acetyl choline in concentrations of $1:10^8$ to $1:10^9$ if previously treated with physostigmine, which augments the sensitivity of the leech muscle about 500 times to acetyl choline. The leech muscle does not respond specifically to acetyl choline but also contracts after exposure to choline, barium, histamine and nicotine. However, these drugs are effective only when applied in quite high concentrations. Furthermore physostigmine augments the sensitivity only to acetyl choline.

In the cat, acetyl choline injected intravenously causes a fall of blood pressure which is accentuated by eserinization of the cat and abolished by atropinisation. This behavior is a valuable means of differentiating acetyl choline from histamine and histamine-like substances. Histamine causes a fall of blood pressure which is not influenced by eserinization or atropinisation.

The efficiency of the skin extraction method used was tested by control experiments. Intracutaneous injections of acetyl choline in various concentrations were made into the abdominal skin of eserinized dogs. It was found that if 1 cc. of $1:10^6$ acetyl choline or 1 microgram was injected within an area of 2 x 2 inches, the weight of the excised skin being 4.5 to 5 grams, the presence of acetyl choline could be shown in the biologic tests.

Results

In man, 8 experiments were made by stimulating the skin with intracutaneous injections of saline, aolan, and histamine; 3 experiments with ultraviolet light; and 2 with hot water. In the blood coming from the stimulated area no acetyl choline could be demonstrated.

In animals, 7 experiments were made with intracutaneous injections of aolan, milk, and histamine; 1 experiment with rough friction of the skin; 3 with hot water; 1 with cold water; and 1 with ultraviolet light. The presence of acetyl choline could not be shown in the outflowing venous blood or in skin extracts.

II. EXPERIMENTS WITH TISSUE FLUIDS OF HUMAN SKIN

a. Histamine wheal fluid in normal human subjects

Method

The tissue fluid obtained by scarification of the human skin after application of vasodilator stimuli was tested for acetyl choline. The technic of collecting the fluid was similar to that of obtaining fluid for dark field examinations

for spirochetes. The scarification lines were made close together over an area of about 1 to 2 square cm. The skin was stretched and pressed until about 0.01 cc. of serum appeared on the surface. The serum was drawn up and measured by a capillary pipette. By taking several samples under the same conditions their biologic effect was proved to be similar.

In human skin wheals were produced by intradermal injection of 0.1 cc. of histamine-physostigmine mixtures (histamine 1:1,000, prostigmine 1:20,000). The tissue liquid obtained from the scarified wheals was tested for acetyl choline on the blood pressure of the cat and on the leech muscle preparation.

Results

Nine such experiments were carried out in normal human skin and the wheal fluid was tested on leech muscle. In 4 of them *definite contractions of the eserinized leech muscle were produced by the tissue liquid* (fig. 1B).

Of 4 blood pressure experiments 2 were positive (fig. 1A). The wheal serum caused a *marked fall of pressure which was enhanced by physostigmine and abolished or weakened by atropine.* In the other 2 experiments the fall before and after atropine was the same, thus indicating the presence of the injected histamine, but the absence of acetyl choline.

Wheal liquids obtained from control wheals containing no histamine (wheals produced by intracutaneous injection of saline, atropine, physostigmine) had no effect on the cat's blood pressure or on the leech muscle.

b. Wheal liquid from skin lesions

Having obtained some evidence of acetyl choline liberation in the skin after injection of histamine, it seemed of interest to determine whether a similar mechanism occurs in spontaneous skin eruptions.

Physostigmine (1:20,000) was injected intradermally into various skin lesions and after scarification the wheal fluid of these lesions was tested for acetyl choline.

In one case of *dry neurodermatitis* (atopic dermatitis) small contractions of the eserinized leech muscle were elicited by fluid from both diseased and normal skin, if the liquid was taken from wheals produced by injection of saline. However a strongly positive response was elicited by fluid from a physostigmine wheal of neurodermatitis skin. In a second case of dry neuro-

dermatitis fluid from wheals of both normal and lichenified skin gave the same response. In a third case the normal skin gave an effect but the diseased skin did not.

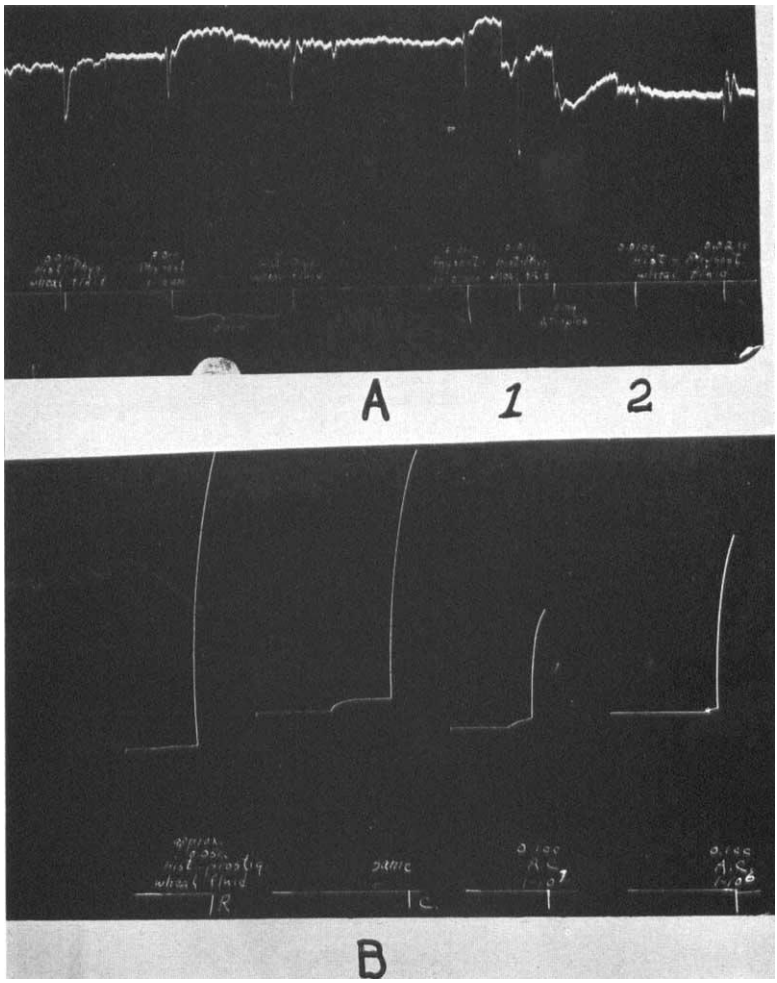

FIG. 1. A. Effect of fluid from histamine-prostigmine wheal on the cat's blood pressure. The depressor effect of the fluid is enhanced after eserinization (1) and destroyed after atropinization (2).

B. Effect of fluid from histamine-prostigmine wheals on the eserinized leech muscle preparation. The first two curves show the contractions caused by wheal liquid from two different persons. The last two curves show the response to acetyl choline 0.01 and 0.1 microgram respectively.

In one case of *dermatitis herpetiformis* the vesicle fluid gave a definite contraction which was stronger if the vesicle content was mixed with eserine than if mixed with saline.

In one case of *pemphigus erythematodes* (Senear-Usher type) a definite contraction was obtained with the contents of a bulla in an early stage of the disease. The response was of the acetyl choline type and could be easily distinguished from the contractions given by strong histamine solutions. In a later stage of the disease the content of bullae did not react.

In one case of *psoriasis* the first sample of serum caused a strong contraction but the leech preparation did not respond to subsequent samples. Such a behavior does not support the acetyl choline nature of the substance causing the contraction.

COMMENT

Conclusive evidence that the contractions elicited in the leech muscle by the histamine wheal fluid are really due to the presence of acetyl choline has not been obtained to date. However, in favor of the acetyl choline nature of the substance are the following experiments:

1. Stronger contractions of the muscle were produced by fluid expressed from wheals raised by physostigmine-histamine mixtures than from those raised by histamine alone.

2. The effect of the tissue serum was diminished or abolished if it was allowed to stand for a time at room temperature. Furthermore, the first sample of tissue serum always had a stronger effect than later samples from the same scarified region. (These findings could be explained by the hydrolysis of acetyl choline by the tissue esterases.)

3. Fluid expressed from wheals raised by intradermal injection of physostigmine alone or of other substances which do not provoke a marked erythematous area gave little or no response.

4. The non-eserinized leech muscle did not respond whereas the eserinized preparation frequently reacted strongly.

5. In two of the blood pressure tests on the cat the wheal serum caused a strong fall which was enhanced by physostigmine and abolished by atropine. In this way acetyl choline could be differentiated from histamine. (If the fall had been caused

be histamine, it would not have been influenced by physostigmine or atropine.)

However these evidences are weakened by the following observations:

1. The leech muscle contraction could not be produced consistently.

2. In two of the blood pressure experiments histamine could be demonstrated in the wheal fluid but acetyl choline could not. The fall before and after atropine was the same.

3. Occasionally contractions of the eserinized leech muscle occurred when it was treated with strong histamine solutions. Although this effect is moderate in relation to the acetyl choline effect and although the nature of the contraction is evidently different from that elicited by acetyl choline and wheal fluids, this observation makes it necessary to be cautious in the interpretation of the experiments.

Therefore further experiments are necessary to determine to what extent the observed contraction of leech muscle is due to acetyl choline or to other contraction-producing substances such as histamine, choline, and potassium. Furthermore, it is necessary to determine what factors are responsible for the fluctuations.

Concerning the preliminary experiments on skin lesions the identification of the substance causing contraction of the leech muscle has not yet been established, and the question whether it is acetyl choline or not must be studied further. Conservatism is specially necessary because of the conclusions drawn by Gollwitzer-Meyer (7) that acetyl choline is liberated after even the slightest stimulus to the skin.

SUMMARY

1. In skin and outflowing venous blood acetyl choline could not be demonstrated after cutaneous stimulation.

2. In liquid obtained by scarification of histamine wheals in human skin a substance was demonstrated which caused contraction of the eserinized leech muscle. Some experiments are presented which speak in favor of the acetyl choline nature of this substance, but the evidence is not conclusive.

3. In individual cases of pathological inflammatory cutaneous

lesions similar effects of the diseased tissue liquid was shown on the leech muscle preparation.

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